

**AMENDMENTS TO THE CLAIMS**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**LISTING OF CLAIMS:**

1-101. (Canceled).

102. (Currently Amended) An isolated nitrile hydratase comprising an  $\alpha$ -subunit and a  $\beta$ -subunit, wherein:

(a) ~~said~~the  $\alpha$ -subunit is ~~a variant of the peptide~~comprises the polypeptide of SEQ ID NO: 1, ~~wherein except that the amino acid sequence of said variant of the peptide~~the polypeptide of SEQ ID NO: 1 is substituted at positions:  
36~~th~~ to Met, ~~or~~  
71~~st~~ to His, ~~or~~  
148~~th~~ to Asp, ~~or~~  
204~~th~~ to Arg, Lys, Trp or Thr, ~~or~~  
148~~th~~ and 204~~th~~ to Asp and Arg respectively, ~~or~~  
36~~th~~, 148~~th~~ and 204~~th~~ to Met, Asp and Arg respectively,  
36 and 126 to Met and Tyr respectively,  
6, 36, and 126 to Met, Thr and Tyr respectively, or  
19, 71, and 126 to His, Val and Tyr respectively; and

(b) the  $\beta$ -subunit comprises the polypeptide of SEQ ID NO: 2.

103-104. (Canceled).

105. (Currently Amended) The nitrile hydratase according to claim ~~103~~102, wherein the  $\beta$ -subunit ~~is~~consists of the amino acid sequence~~polypeptide~~ of SEQ ID NO: 2.

106. (Currently Amended) ~~The~~ An isolated nitrile hydratase comprising an  $\alpha$ -subunit and a  $\beta$ -subunit, according to claim 102~~wherein:~~

(a)(i) the  $\alpha$ -subunit comprises the polypeptide of SEQ ID NO: 1, except that the amino acid sequence of the polypeptide of SEQ ID NO: 1 is substituted, wherein the  $\beta$ -

~~subunit is a variant of the peptide of SEQ ID NO: 2, wherein the  $\alpha$ -subunit is substituted amino acid at positions 36<sup>th</sup>, 148<sup>th</sup> and 204<sup>th</sup> of SEQ ID NO: 1 to Met, Asp and Arg respectively, and (ii) the  $\beta$ -subunit comprises the polypeptide of SEQ ID NO: 2, except that the amino acid sequence of the polypeptide of SEQ ID NO: 2 is substituted amino acid at positions 41<sup>st</sup>, 51<sup>st</sup> and 108<sup>th</sup> of SEQ ID NO: 2 to Ile, Val, and Asp respectively;~~

(b)(i) the  $\alpha$ -subunit comprises the polypeptide of SEQ ID NO: 1, except that the amino acid sequence of the polypeptide of SEQ ID NO: 1 is substituted at positions 6, 36 and 126 of SEQ ID NO: 1 to Thr, Met and Tyr respectively, and (ii) the  $\beta$ -subunit comprises the polypeptide of SEQ ID NO: 2, except that the amino acid sequence of the polypeptide of SEQ ID NO: 2 is substituted at positions 10, 118, and 200 of SEQ ID NO: 2 to Asp, Val and Glu respectively;

(c)(i) the  $\alpha$ -subunit comprises the polypeptide of SEQ ID NO: 1, except that the amino acid sequence of the polypeptide of SEQ ID NO: 1 is substituted at positions 19, 71 and 126 of SEQ ID NO: 1 to Val, His and Tyr respectively, and (ii) the  $\beta$ -subunit comprises the polypeptide of SEQ ID NO: 2, except that the amino acid sequence of the polypeptide of SEQ ID NO: 2 is substituted at positions 37, 108, and 200 of SEQ ID NO: 2 to Leu, Asp, and Glu respectively;

(d)(i) the  $\alpha$ -subunit comprises the polypeptide of SEQ ID NO: 1, except that the amino acid sequence of the polypeptide of SEQ ID NO: 1 is substituted at positions 19, 71 and 126 of SEQ ID NO: 1 to Val, His and Tyr respectively, and (ii) the  $\beta$ -subunit comprises the polypeptide of SEQ ID NO: 2, except that the amino acid sequence of the polypeptide of SEQ ID NO: 2 is substituted at positions 37, 108, and 200 of SEQ ID NO: 2 to Val, Asp, and Glu respectively; or

(e)(i) the  $\alpha$ -subunit comprises the polypeptide of SEQ ID NO: 1, except that the amino acid sequence of the polypeptide of SEQ ID NO: 1 is substituted at positions 148 and 204 of SEQ ID NO: 1 to Asp and Arg respectively, and (ii) the  $\beta$ -subunit comprises the polypeptide of SEQ ID NO: 2, except that the amino acid sequence of the polypeptide of SEQ ID NO: 2 is substituted at positions 108, and 200 of SEQ ID NO: 2 to Asp, and Glu respectively.

109. (Currently Amended) An isolated nitrile hydratase comprising an  $\alpha$ -subunit and a  $\beta$ -subunit, wherein:

(a) the  $\alpha$ -subunit comprises the polypeptide of SEQ ID NO: 1; and

(b) the  $\beta$ -subunit is a variant of the peptide comprises the polypeptide of SEQ ID NO: 2, wherein said except that the amino acid sequence of variant the polypeptide of the SEQ ID NO: 2 is substituted at positions:

10<sup>th</sup> to Asp, Glu, Trp, Gly, Tyr or Cys,-or

32<sup>nd</sup> to Gly,-or

37<sup>th</sup> to Thr, Ala, Leu, Ile or Val,-or

41<sup>st</sup> to Glu, Thr, Ala, Leu, Ile, or Val,Val,-or

46<sup>th</sup> to Gly, Tyr, Leu, Lys or Asp,-or

48<sup>th</sup> to Gly, Ala, Val, Ser, Thr or Arg,-or

51<sup>st</sup> to Ala or Val,-or

72<sup>nd</sup> to Phe,-or

118<sup>th</sup> to Ala, Leu, Ile or Val,-or

127<sup>th</sup> to Ala, Val or Ser,-or

146<sup>th</sup> to Gly,-or

160<sup>th</sup> to Leu or Trp,-or

186<sup>th</sup> to Glu, Asp, Lys, Arg, Asn, Ser or Gly,-or

217<sup>th</sup> to Gly,-or

160<sup>th</sup> and 186<sup>th</sup> to Trp and Arg respectively,-or

127<sup>th</sup>, 160<sup>th</sup> and 186<sup>th</sup> to Ser, Trp and Arg respectively,

51 and 108 to Val and Asp respectively,

118 and 200 to Val and Glu respectively,

10, 118 and 200 to Asp, Val and Glu respectively,

37, 108 and 200 to Leu, Asp and Glu respectively,

37, 108 and 200 to Val, Asp and Glu respectively,

41, 51 and 109 to Ile, Val and Asp respectively,

46, 108 and 212 to Lys, Arg and Tyr respectively, or

48, 108 and 212 to Val, Arg and Tyr respectively.

110. (Canceled).

111. (Currently Amended) The nitrile hydratase according to claim 109, wherein the  $\alpha$ -subunit ~~has the amino acid sequence consists of the polypeptide of~~ SEQ ID NO: 1.

112. (Canceled).

113. (Currently Amended) An isolated nitrile hydratase comprising an  $\alpha$ -subunit and a  $\beta$ -subunit, wherein:

~~(a)(i) the  $\alpha$ -subunit is a variant of the peptide comprises the polypeptide of~~ SEQ ID NO: 1, ~~wherein the  $\beta$ -subunit is a variant of the peptide of~~ SEQ ID NO: 2, ~~wherein amino acid of the  $\alpha$ -subunit except that the amino acid sequence of the polypeptide of~~ SEQ ID NO: 1 is substituted at positions 6<sup>th</sup>, 19<sup>th</sup> and 126<sup>th</sup> of SEQ ID NO: 1 to Thr, Val and Tyr respectively, and ~~(ii) amino acid of the  $\beta$ -subunit comprises the polypeptide of~~ SEQ ID NO: 2, ~~except that the amino acid sequence of the polypeptide of~~ SEQ ID NO: 2 is substituted at positions 46<sup>th</sup>, 108<sup>th</sup> and 212<sup>th</sup> of SEQ ID NO: 2 to Lys, Arg and Tyr respectively; or

~~(b)(i) the amino acid of the  $\alpha$ -subunit comprises the polypeptide of~~ SEQ ID NO: 1, ~~except that the amino acid sequence of the polypeptide of~~ SEQ ID NO: 1 is substituted at positions 6<sup>th</sup>, 19<sup>th</sup> and 126<sup>th</sup> of SEQ ID NO: 1 to Thr, Val and Tyr respectively, and ~~(ii) amino acid of the  $\beta$ -subunit comprises the polypeptide of~~ SEQ ID NO: 2, ~~except that the amino acid sequence of the polypeptide of~~ SEQ ID NO: 2 is substituted at positions 48<sup>th</sup>, 108<sup>th</sup> and 212<sup>th</sup> of SEQ ID NO: 2 to Val, Arg and Tyr respectively, or

~~(c)(i) the amino acid of the  $\alpha$ -subunit comprises the polypeptide of~~ SEQ ID NO: 1, ~~except that the amino acid sequence of the polypeptide of~~ SEQ ID NO: 1 is substituted at positions 6<sup>th</sup>, 19<sup>th</sup> and 126<sup>th</sup> of SEQ ID NO: 1 to Ala, Val and Tyr respectively, and ~~(ii) amino acid of the  $\beta$ -subunit comprises the polypeptide of~~ SEQ ID NO: 2, ~~except that the amino acid sequence of the polypeptide of~~ SEQ ID NO: 2 is substituted at positions 127<sup>th</sup>, 160<sup>th</sup> and 186<sup>th</sup> of SEQ ID NO: 2 to Ser, Trp and Arg respectively.

114. (Previously Presented) An isolated host cell comprising the nitrile hydratase of claim 102.

115. (Previously Presented) An isolated host cell comprising the nitrile hydratase of claim 109.

116. (Previously Presented) An isolated host cell comprising the nitrile hydratase of claim 113.

117. (New) An isolated host cell comprising the nitrile hydratase of claim 106.

118. (New) An isolated nitrile hydratase comprising an  $\alpha$ -subunit and a  $\beta$ -subunit, wherein:

(a) the  $\alpha$ -subunit comprises the polypeptide of SEQ ID NO: 1, except that the amino acid sequence of the polypeptide of SEQ ID NO: 1 is substituted at any one or more of positions 6, 19, 36, 71, 126, 148, and 204 by another amino acid; and

(b) the  $\beta$ -subunit comprises the polypeptide of SEQ ID NO: 2, except that the amino acid sequence of the polypeptide of SEQ ID NO: 2 is substituted at any one or more of positions 10, 20, 21, 32, 37, 41, 46, 48, 51, 72, 108, 118, 127, 146, 160, 186, 200, 212, and 217 by another amino acid.

119. (New) An isolated host cell comprising the nitrile hydratase of claim 118.